

## Single cell analysis of commitment in early embryogenesis

JANET HEASMAN, ALISON SNAPE, JIM SMITH AND  
CHRISTOPHER C. WYLIE

*Department of Anatomy, St George's Hospital Medical School, Cranmer Terrace,  
London, SW17 0RE, U.K.*

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### SUMMARY

Fate maps of amphibian embryos tell us the destination of certain areas at later stages of development. After studying Vogt's fate maps, Spemann wrote (in 1938) that "the question which at once calls for an answer is whether this pattern of presumptive primordia in the beginning gastrula is the expression of a real difference of these parts, whether they are already more or less predestined or 'determined' for their ultimate fate, or whether they are still indifferent and will not receive their determination until a later time." Until recently answers to this question have relied upon explant experiments, which indicate that by the late blastula stage ectoderm, mesoderm and endoderm regions are distinctly determined. By using a method involving single cell labelling and transplantation, it is now possible to pinpoint more accurately the time during early embryogenesis at which individual blastomeres become committed. In the vegetal pole, determination towards endoderm is a gradual process beginning during the middle blastula stage (stage 8) and completed by the beginning of gastrulation (stage 10). This method offers the possibilities of comparing the committed and the uncommitted state and studying at a molecular level the mechanisms of cell determination.

### INTRODUCTION

The analysis of early development of embryos at a single cell level is not a new pursuit, although until recently interest has concentrated on classes other than the Amphibia. For example, studies on the cell lineages of mouse (Gardner, 1977), insect (Garcia-Bellido, Lawrence & Morata, 1979), leech (Stent, Weisblat, Blair & Jackson, 1982), and Nematodes (Sulston & Horowitz, 1977) predate those to be described here in *Xenopus* embryos. Work on such diverse animals as mouse and insects has provided very different insights into the process of determination, by which cells and their progeny become committed to particular pathways of development. In each case, the experimental approach is dictated by the advantages and limitations of each embryo type, and by the techniques available for their manipulation. Broadly speaking, in mouse and amphibia the cellular approach has been adopted, while in *Drosophila* the combination of molecular genetics and cellular studies has yielded the clearest picture so far of mechanisms of determination.

*Key words:* commitment, specification, determination, induction, TRITC, amphibia.

## ANALYSIS OF CELL COMMITMENT IN MOUSE EMBRYOS

In mouse embryos, a single cell analysis of determination has involved the injection of cells of the preimplantation embryo into the blastocoels of host blastocysts (Gardner, 1968). The donor cells have been labelled in a number of ways (reviewed by Gardner, 1984), so that chimaerism could be detected in external and/or internal tissues of the foetus. The sequence of commitment in the preimplantation embryo appears to be as shown in Fig. 1. It seems that a series of binary decisions establishes the primitive ectoderm as the source of all foetal tissue, and sets aside the cell lineages of the extraembryonic membranes. The timing and sequence of further segregation of the primitive ectoderm into the foetal tissues is obscure, largely because these steps occur after the embryo has implanted and postimplantation cells will not integrate into blastocyst-stage host embryos.

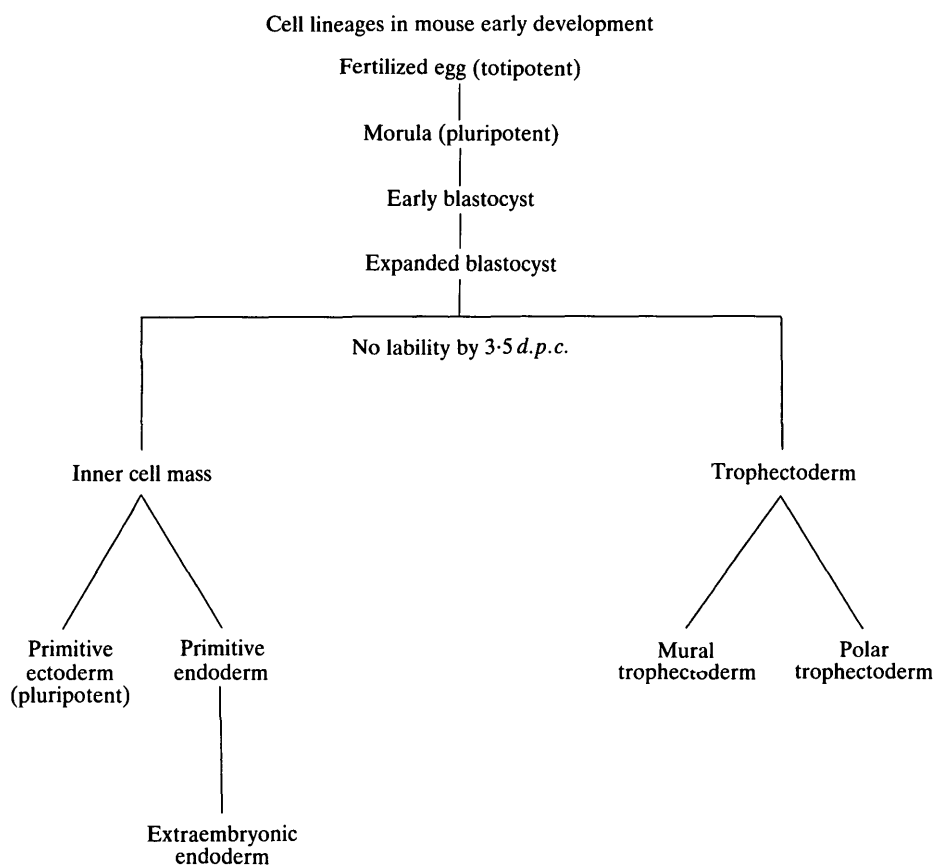


Fig. 1. Cell commitment in the preimplantation mouse embryo.

A second line of enquiry has been to identify changes in the properties of cells *e.g.* in adhesion, division rate, polarity or morphology which might be indicative or even operational in determination (Mintz, 1965; Tarkowski & Wroblewska, 1967). In the morula of early blastocyst two subpopulations have been distinguished, consisting of large polar surface phenotype and smaller inner cells with characteristically different microvillar patterns (Johnson & Ziomek, 1982). Both types of blastomere are pluripotent at this stage (Ziomek, Johnson & Handyside, 1982). Individual cells have been labelled and aggregated with 15 unlabelled 16-cell blastomeres, either in an inside or outside position. Results showed that polar cells normally form trophectoderm, even if placed in an ectopic position, while the smaller apolar cells were found in all combinations of inner cell mass and trophectoderm. This suggests that the polar phenotype is important in determining the fate of the outside cells (Ziomek & Johnson, 1982).

#### STUDIES ON CELL COMMITMENT IN *DROSOPHILA*

In view of the long history of genetics and mutation studies in *Drosophila* it is perhaps not surprising that the first clues to the mechanism of determination at a genetic level should come from insect work. The recently accumulated evidence that the segmentation and homeotic genes act as regulatory genes controlling the pattern and form of each segment in adult flies will not be reviewed here (Lewis, 1978; Nüsslein-Volhard, Wieschaus & Jürgens, 1982; McGinnis *et al.* 1984). However a number of aspects may be relevant to amphibian studies.

Firstly, the compartments in which these genes act were first recognized in insects by cellular studies, in which individual blastoderm cells were marked by X-ray-induced somatic mutation techniques, to produce visibly identifiable 'polyclones' on the adult surface (Garcia-Bellido *et al.* 1979). Initially the restriction is to particular body segments (Chan & Gehring, 1971; Lawrence & Morata, 1977) (by the cellular blastoderm stage). Each thoracic and abdominal segment is formed by a subdivision of the blastoderm into evenly spaced bands, each three or four cells wide (Lohs-Schardin, Cremer & Nüsslein-Volhard, 1979). A cell lineage restriction between neighbouring segments occurs at or soon after this stage (Wieschaus & Gehring, 1976), and is followed by determinative steps specifying anterior/posterior and dorsal/ventral compartments (Garcia-Bellido *et al.* 1979). Thus a series of binary decisions gradually restricts groups of cells to particular sites in the developing insect.

Genetic and molecular studies of the pattern of expression of homeotic and segmentation genes have substantiated the compartment theory, (Lewis, 1978; Kauffman, 1980; Struhl, 1982). For example, *in situ* hybridization experiments show that transcripts from the segmentation gene, *fushi tarazu*, appear in a segmented fashion in the blastoderm stage, and are distributed in bands of three to five cells width followed by three to five unlabelled cells. Interestingly, transcripts appear in this segmented fashion even before the blastoderm becomes cellularized (Hafen,

Kuriowa & Gehring, 1984) suggesting that cytoplasmic localizations of the egg interact with cleavage nuclei. Embryos homozygous for mutations in this gene possess only half the segments of wild-type embryos, and die in late embryogenesis.

Both segmentation and homeotic genes are responsible for regional specification in *Drosophila* and not for cell type determination. As yet, no genes have been identified in vertebrates or invertebrates which commit cells and their progeny to specific cell types.

Perhaps the most exciting aspect of the homeotic gene story is the evidence that a DNA sequence (the homeobox sequence) common to several homeotic genes of *Drosophila* is also present in the genomes of worms, frogs, birds, mice and man (McGinnis *et al.* 1984; Carrasco *et al.*, 1984). Furthermore transcripts from this sequence are developmentally regulated in *Xenopus* embryos, appearing first at the late gastrula stage (Carrasco, McGinnis, Gehring & de Robertis, 1984). This result suggests that developmentally important genes may be recognized in other species like *Drosophila* and their mode of action studied in the more amenable *Xenopus* embryo. At the moment we know very little about the establishment of metameric segmentation in *Xenopus*. There is a suggestion that regulation may begin at the blastula stage, when heat shocks occurring in a sensitive period before midgastrula stage, result in abnormal somitogenesis (Elsdale & Pearson, 1979).

#### ANALYSIS OF CELL COMMITMENT IN *XENOPUS* EMBRYOS USING SINGLE CELL LABELLING

Although amphibian embryos have been a favourite experimental animal for studies of early development for nearly a hundred years, the mechanisms underlying the formation of a three-dimensional tadpole and adult frog from a fertilized egg remain obscure.

However important advances have been made including the following:

- 1) Accurate fate maps are available (Vogt, 1929; Keller, 1975, 1976). These maps give information about the future position and germ layer of each part of the late blastula and early gastrula. This allows the investigator to choose a particular region of the early embryo and knowing its prospective fate, ask the question, when during early development do the cells here lose their ability to enter any other cell lineage?
- 2) The states of commitment of pieces of tissue from amphibian embryos have been tested using two main operational definitions of determination. In the first, unmarked pieces of tissue have been explanted to ectopic sites in a host embryo and the question asked whether they develop according to their new site, in which case they are not determined, or according to their site of origin, in which case they are determined. Classical experiments of this sort in urodele embryos (Mangold, 1923) show that prospective ectodermal pieces of the early gastrula are not determined and will form mesoderm and endoderm when pushed into the blastocoel of host embryos of a different species at the same stage of development.

The second more popular method of studying the state of commitment of pieces of embryonic tissue has been to isolate parts and study the range of tissues into which the piece develops either in salt solution *in vitro* (Holtfreter 1931) or in the cavities of older host larvae – the coelomic cavity (Holtfreter, 1925, 1929) or eye cavity (Kusche, 1929). The results of these classical studies on early gastrulae were summarized by Spemann in the following way: 'Its three main regions, the germ layers, are already determined toward the ectodermal, mesodermal and endodermal organs which normally issue from them, the two latter even in detail. The material for the two ectodermal tissues, neural tube and epidermis, seems to be as yet indifferent or, certainly, in an extremely labile state of determination' (Spemann, 1938). Since Spemann's time, these results have largely been confirmed and extended (for review see Slack, 1983). In particular the property of self differentiation has been studied at the molecular level with regards to  $\alpha$ -actin gene transcription. It has been shown that the isolated equatorial region of the midblastula, which is fated to become mesoderm, switches on actin gene transcription at approximately the same time as occurs in the intact embryo (Gurdon, Brennan, Fairman & Mohun, 1984). This type of operation tells us about the self-determination capacity of embryonic tissue. However it suffers from the drawback that isolation from the embryo results in a loss of tissue mass and of normal cell interactions both of which may reduce the range of options open to the piece. Thus the full developmental capacity of the isolated parts may not be tested. If the central aim is to be able to recognize the sequence of restriction which cells undergo to develop along a particular pathway, it is important not to provide them with artificial restrictions which may confuse the result.

One way of reducing the problem is to take single cells with the same prospective fates from progressively older embryos and transplant them to the same site in hosts of one particular stage of development.

#### *The experimental design for testing the state of commitment of single cells*

We have recently attempted this using individual blastomeres taken from the vegetal pole of *Xenopus* blastulae and gastrulae and transplanting them to the blastocoels of late blastula hosts (Fig. 2) (Heasman, Wylie, Hausen & Smith, 1984). Vegetal pole blastomeres were chosen firstly because they are easy to isolate in an accurate and reproducible manner, and secondly, because the prospective fate of this region is uncomplicated. We checked the normal fate map at the vegetal pole by injecting individual cells of early and midblastulae and early gastrulae with HRP and examining the distribution of label in the embryos at the tailbud stage. While vegetal blastomeres from the early blastula stage contribute progeny to all three germ layers, those from midblastula and early gastrula contribute to endoderm only.

The experimental design used here to study the state of commitment of these vegetal cells (Fig. 2), depends upon the donor blastomere being labelled, so that its progeny can be detected in the host's tissues. The label we use is tetramethyl

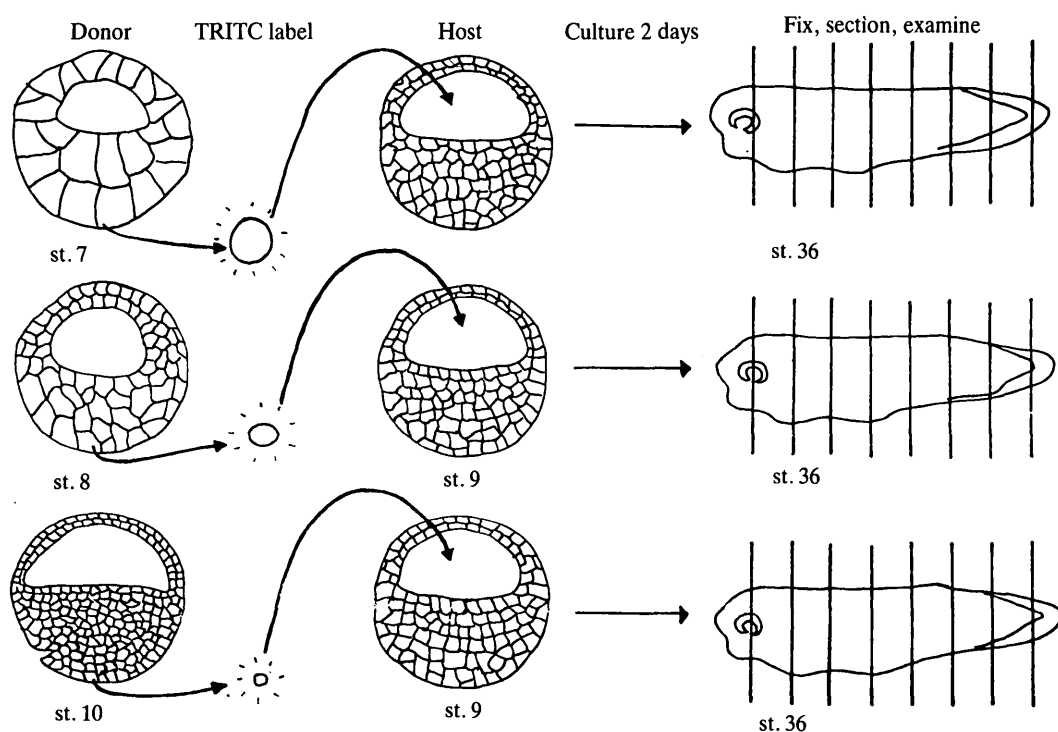


Fig. 2. The design of the experiment to test the state of commitment of cells from the vegetal pole of blastulae and gastrulae. Individual cells were isolated, labelled by immersion in medium containing TRITC, and one labelled cell was placed in each host blastocoel.

rhodamine isothiocyanate (TRITC) (Nordic), which has the advantage of not requiring injection. It is simply taken up by cells which are immersed in medium or buffer containing the dye. The concentration of the dye is quite critical, high concentrations being toxic. As TRITC is not very soluble (we dissolve it in 2 %  $\text{NaHCO}_3$ ), the amount going into the solution may vary. We standardize each TRITC batch to the absorption spectrum obtained from the first batch. The concentration used is  $100 \mu\text{g ml}^{-1}$  in medium containing 10 % FCS. We have tested by three different methods whether the label is cell autonomous (Heasman *et al.* 1984) and are satisfied that this is the case. Brightly labelled cells are occasionally surrounded by a halo of weak fluorescence, but this is not cell specific and can be distinguished very easily from positive labelling. We do not know the cause of this

Fig. 3. Labelled progeny of a single vegetal pole blastomere of an early blastula embryo implanted into the blastocoel of a late blastula host. Labelled cells are found in this section of the host at stage 37, in the neural tube, notochord and somite. The inset black rectangle indicates the area of the larva shown in the fluorescent image. The white arrow points to labelled myofibril bundles in the somite cells, demonstrating their state of differentiation. Bar =  $5 \mu\text{m}$ .

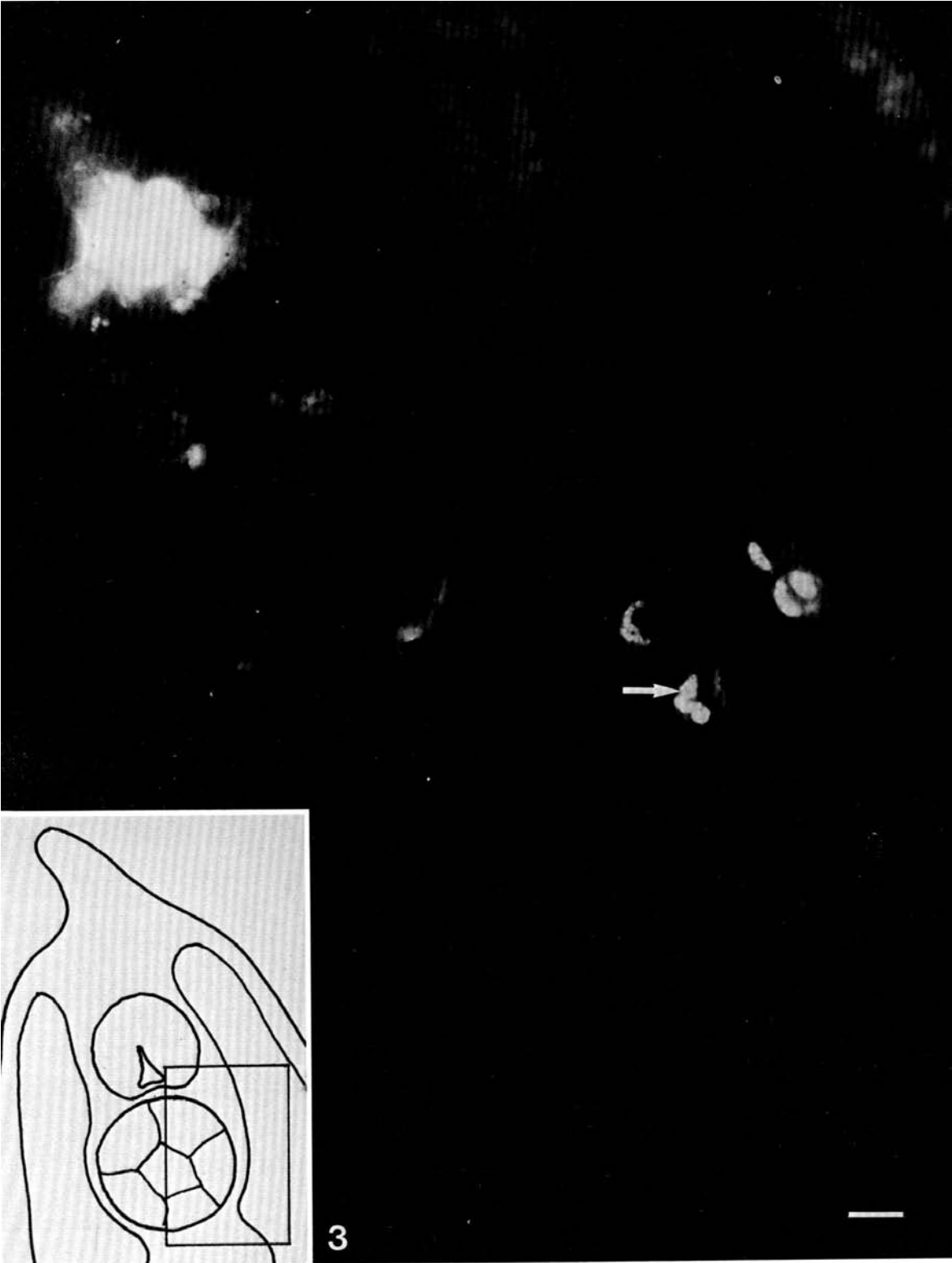


Fig. 3

low-level fluorescence, it may be due to reflected light from surrounding cells, or to low-level leakage from the labelled donor cell and its progeny.

Labelled blastomeres continue to divide, at a rate comparable with that of unlabelled cells, and when transplanted into blastocoels of host embryos, can take a normal part in development. Fig. 3 shows a section through a host which was fixed at the late tailbud stage (stage 36–37), when some overt germ layer differentiation has occurred. Labelled cells are visible in both ectodermal and mesodermal derivatives (neural tube, notochord and somite). The cells in the somite contain bundles of myofibrils (white arrow), indicating that they are differentiating in concert with the rest of the somite. Often, however, morphological differentiation is not so obvious, and we make the assumption that position of labelled progeny in particular germ layers indicates their commitment to those layers. Unfortunately, germ-layer-specific markers are not yet available to test this. We fix hosts at stage 36, late tailbud stage, when the three germ layers can be distinguished clearly, and when the number of serial 10  $\mu\text{m}$  sections per embryo to be examined is manageable. We only fix embryos which have a normal external appearance (approximately 50 % per experiment); all abnormal or slightly abnormal specimens are discarded.

#### *Changes in the state of commitment of vegetal pole cells*

When early blastula vegetal pole cells are transplanted in this way, the progeny of each implanted cell are found in all three germ layers of hosts (Fig. 4). So far, we have not counted the proportions of cells in each germ layer, but have taken the view that even one cell in a particular tissue indicates the potential exists for differentiation into that tissue type. In general however, several cells are found either running longitudinally as a clone through many serial sections, or in a cluster in a few adjacent sections.

Throughout the blastula stages, we find there is a gradual restriction in the ability of transplanted cells to enter all germ layers (Table 1 and Fig. 5), so that by the late blastula stage (stage 9), 82 % of transferred cells have progeny only in endoderm, and by the early gastrula stage all progeny are found in endoderm.

The data given in Table 1 is accumulated from six separate experiments, using different batches of embryos. However, we are confident that variations among the batches of eggs are not a contributing factor in the pattern as within one experiment, using a single batch of eggs as donors at early-, mid- and late-blastula stages, we find the same result (Table 2).

From these experiments the conclusions are twofold: Firstly that it is not until the early gastrula stage that all superficial vegetal pole cells are committed to their normal fate of forming endoderm and secondly, that commitment to endoderm is not an instant phenomenon, where a switch is thrown throughout the area at one time.

To explore this phenomenon of gradual restriction further, we asked whether commitment occurs autonomously or whether continued cell interaction is required. Fig. 6 shows the design of the experiment. We isolated mid- and late-blastula vegetal pole cells (in separate experiments) labelled them and cultured

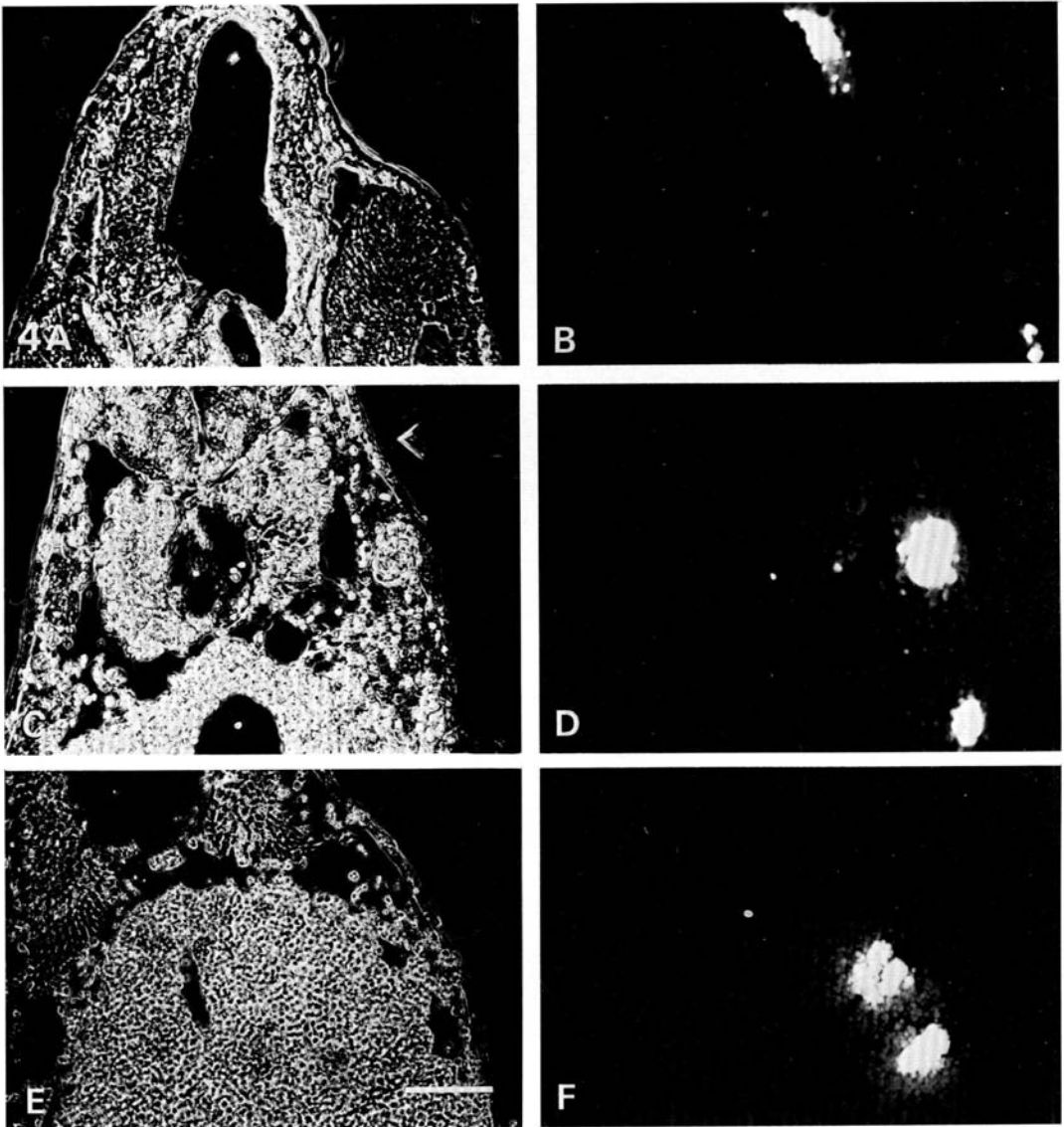


Fig. 4. Phase and fluorescent pairs of photographs to show the germ layer derivatives in which labelled cells are found. (A,B) Progeny of one labelled early blastula vegetal pole cell are found in ectodermal derivatives, the forebrain and epidermis, of a section of a late tailbud host embryo. (C–F) Progeny of labelled midblastula vegetal pole cells are found here in mesodermal (C,D) and endodermal germ layers (E,F) of host embryos. In Fig. 4C,D one cell is labelled in the non-differentiated mesoderm between the somite and epidermis, while one labelled cell lies in the lateral plate mesoderm, between the gut and epidermis. Bar = 100  $\mu$ m.

them for 4–8 h in buffered saline. Those clumps of cells which had resulted from three or four divisions of the donor cell were dissociated in 67 mM-phosphate buffer and each cell was transplanted into a late blastula host. The rest of the experimental

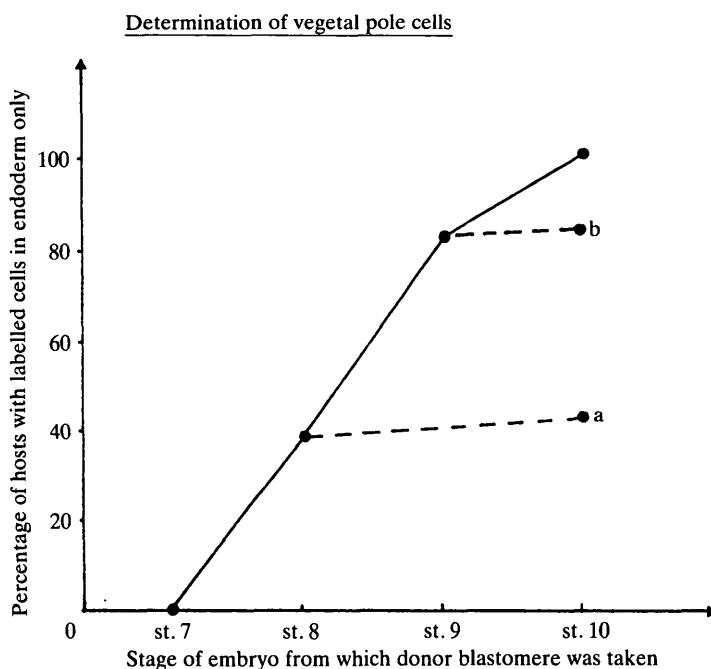


Fig. 5. Embryonic stage and the state of commitment of vegetal pole cells, using the test of commitment described in Fig. 2. A committed donor cell gives labelled progeny in endoderm only. Unbroken lines represent results from Table 1; dashed lines represent the results from Table 3, where isolated blastomeres were cultured from stage 8 – stage 10 before transplanting (line a), and stage 9 – stage 11 before transplanting (line b).

Table 1. *Compiled results of superficial vegetal pole cell transfers*

Source of donor cells	Number of hosts with labelled cells	The number of hosts containing labelled cells in the following combinations of germ layers						
		end mes ect	end only	end mes only	end ect only	mes ect only	mes only	ect only
st. 7 early blastula	8	8	0	0	0	0	0	0
st. 8 mid blastula	66	19	26	17	1	3	0	0
st. 9 late blastula	67	2	55	8	0	0	2	0
st. 10 early gastrula	22	0	22	0	0	0	0	0

procedure was as before. We find that the progeny of cultured donor cells become no more committed *in vitro* than their ancestors, despite the fact that those left in the embryo do so (Table 3 and Fig. 5).

Table 2. Results of superficial vegetal pole cell transfers using embryos from one female

Source of donor cells	Number of hosts with labelled cells	The number of hosts containing labelled cells in the following combinations of germ layers						
		end mes ect	end only	end mes only	end ect only	mes ect only	mes only	ect only
st. 7	8	8	0	0	0	0	0	0
st. 8	22	5	11	6	0	0	0	0
st. 9	20	1	17	2	0	0	0	0

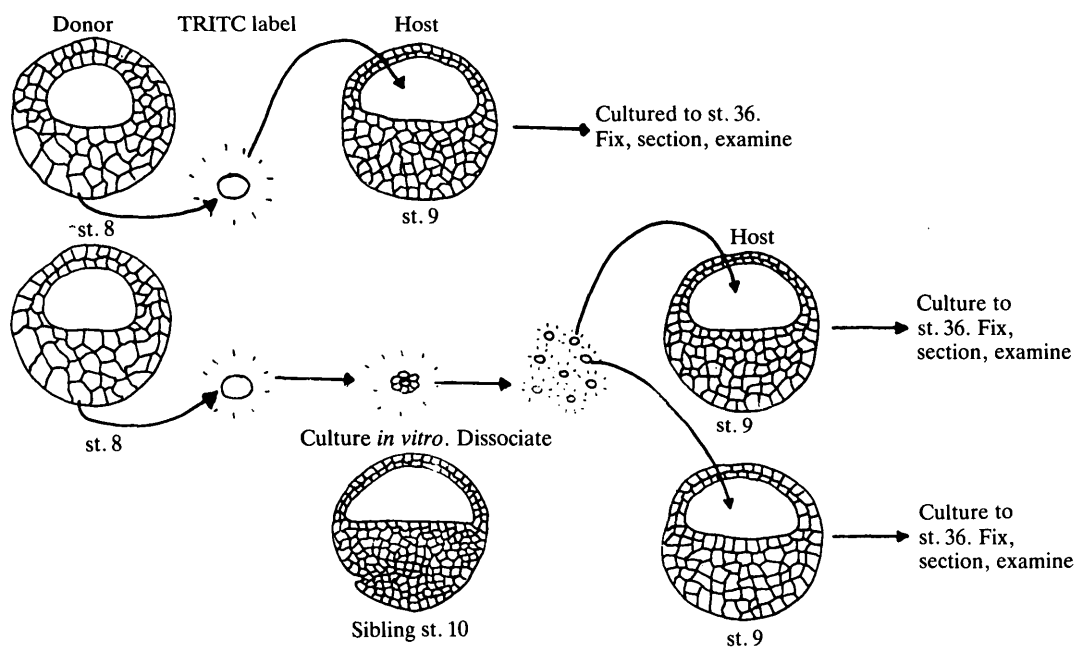


Fig. 6. The experimental design to test whether commitment is a cell autonomous event. The state of commitment of cells isolated from stage-8 blastulae and transplanted directly, is compared to that of stage-8 cells cultured *in vitro* until sibling embryos reach stage 10, and then transferred to hosts.

An important control for this experiment is to compare the division rate of cells *in vitro* with that in equivalent cells of intact embryos. To do this we isolated late-blastula vegetal pole cells and cultured them until sibling embryos reached the midgastrula stage. We then took cells from the yolk plug of the gastrula siblings and compared the size of the two groups of cells. Fig. 7 shows that they are the same size, indicating that culture conditions do not affect division rate. The inability of

Table 3. *Compiled results on the effect of in vitro culture on the determination of vegetal pole cells*

Source of donor cells	Number of hosts with labelled cells	The number of hosts containing labelled cells in the following combinations of germ layers						
		end mes ect	end only	end mes only	end ect only	mes ect only	mes only	ect only
stage 8 single cells cultured	79	10	34	19	3	7	6	0
stage 9 single cells cultured	31	1	26	4	0	0	0	0
stage 8 whole area IV cultured	27	1	23	2	1	0	0	0

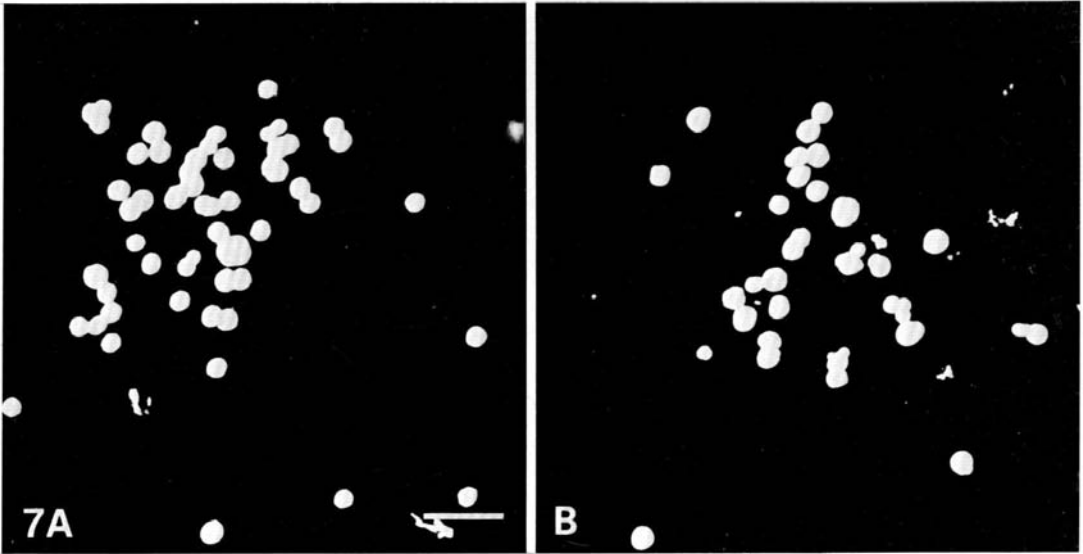


Fig. 7. The division rate of blastomeres in culture compared with that in the intact embryo. In Fig. 7A cells were isolated from the vegetal pole of late blastulae and cultured in saline until sibling intact embryos reached the mid-gastrula stage. The clumps of cells were dissociated. In Fig. 7B cells were isolated directly from the yolk plug of mid-gastrula embryos. Notice the cultured cells and newly isolated cells are of the same size, indicating that cell division continues at the normal rate *in vitro*. Bar = 250µm.

mid- and late-blastula cells to become further committed *in vitro* cannot be due to them not dividing on schedule. Interestingly, when we culture the entire vegetal mass (Nieuwkoop area IV) from the midblastula to early-gastrula stage, and then

dissociate and transplant single cells from that mass, we find that each cell has become committed in culture to form endoderm (Table 3). This suggests that vegetal pole cells require continued cell interaction for commitment to progress, and that this interaction continues to be required once the process has started.

The sequence of steps by which cells which are pluripotent at the early blastula stage develop into groups of 'endoderm only' cells by the early gastrula stage is still unclear. The results suggest that vegetal cells may lose competence to form ectoderm before mesodermal competence is lost.

We are presently studying the possible sequences by which cells become committed to one germ layer. Two alternatives are: 1) cells are either 'on' or 'off' with regards to commitment to form endoderm. The number of cells in the 'on' state increases throughout the blastula stages to reach 100 % by the early gastrula stage. 2) All the cells at the vegetal pole are the same but they all progress through an initially reversible stage of commitment. The ratio of forward and backward rate constants for this first step,  $K_1/K_2$ , increases during the blastula stage so that commitment is irreversible by stage 10. This model is favoured as a mechanism for the differentiation of a variety of mammalian lineages (Bennett, 1983). We are currently trying to discriminate between the two possibilities experimentally.

#### *Commitment in the animal pole cells*

Similar experiments to those described above have been tried using cells from the animal pole of blastula and gastrula stages (Fig. 8 and Table 4). The normal fate of the subcortical animal pole cells we use is to enter ectodermal lineages only (Keller, 1975, 1976). Preliminary results suggest that a similar sequence of gradual restriction occurs at the animal pole as that happening in vegetal blastomeres (Snape *et al.* unpublished data). At the early blastula stage, animal pole cells are pluripotent. However, after this stage, experiments are made more complex because two phenomena, commitment and induction, appear to be involved. When mid and late blastula animal pole cells are placed in stage-8 to -9 hosts none of the progeny are

Table 4. *Compiled results of subcortical animal pole cell transfers*

Source of donor cells	Number of hosts with labelled cells	The number of hosts containing labelled cells in the following combinations of germ layers						
		end mes ect	end only	end mes only	end ect only	mes ect only	mes only	ect only
st. 7 early blastula	28	25	1	2	0	0	0	0
st. 8 mid blastula	20	1	1	7	0	1	10	0
st. 9 late blastula	45	4	16	6	1	4	14	0

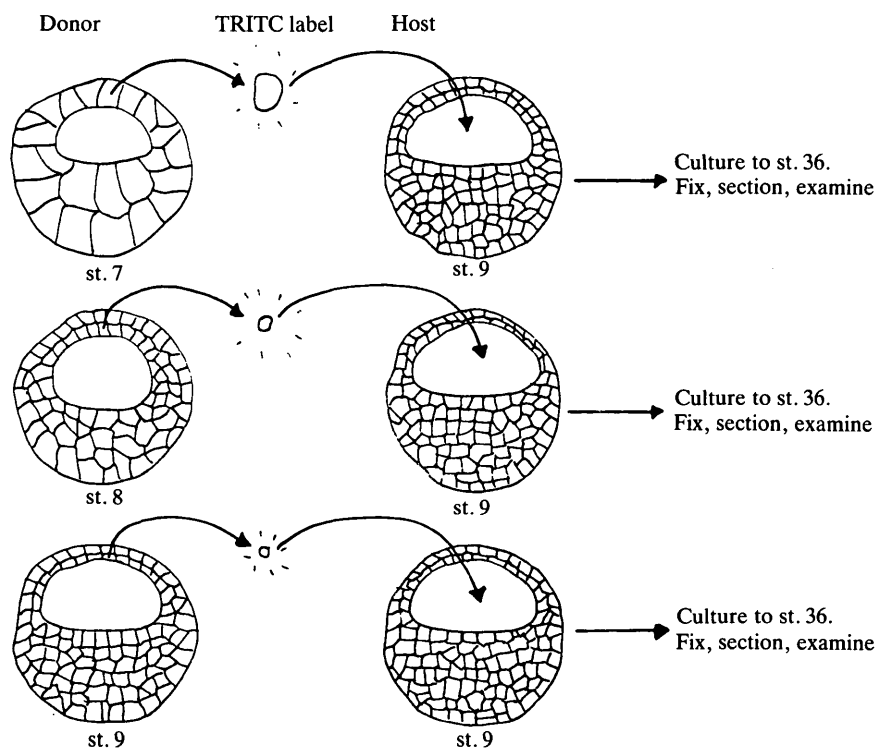


Fig. 8. The experimental design to test commitment of subcortical cells from the animal pole of blastula stage embryos.

found in ectoderm only. The majority form either clones in endoderm only or mesoderm only. The recombination experiments of Nieuwkoop (reviewed by Nakamura, 1978) have shown that the vegetal mass of embryos of this age has an inductive effect on animal pole pieces of tissue. This phenomenon is evident when vegetal masses are taken from early or late blastulae but is absent when they are taken from the early gastrula stage. As, in our experiments, injected donor cells generally fall to the floor of the blastocoel on to the vegetal mass, they are likely to be influenced by this inducing effect. We tested this possibility by injecting late blastula donor cells into midgastrula hosts (non-inducing according to Nieuwkoop and Nakamura) as well as the midblastula hosts (Table 5). The animal pole cells formed endoderm and mesoderm, as before, in 'inducing' hosts suggesting that a vegetalizing rather than a mesodermalizing influence is at work (Nieuwkoop & Ubbels, 1972). In a non-inducing environment 34 % of the donor cells were committed to ectoderm only. This suggests that a gradual process of commitment occurs in the animal pole. Early blastula cells are pluripotent and are not competent to respond to inductive influences. Mid- and late-blastula cells are gradually becoming committed. However this phase of commitment is reversible, and its direction can be changed by bringing the cells into abnormally close contact with vegetal blastomeres. In natural development this contact is prevented by the presence of the

Table 5. Results of transfers of stage-9 animal pole cells into hosts of different stages

Host age at time of transfer	Number of hosts with labelled cells	The number of hosts containing labelled cells in the following combinations of germ layers						
		end mes ect	end only	end mes only	end ect only	mes ect only	mes only	ect only
stage 8 hosts	18	3	11	2	1	1	0	0
stage 11	29	1	4	3	4	7	0	10

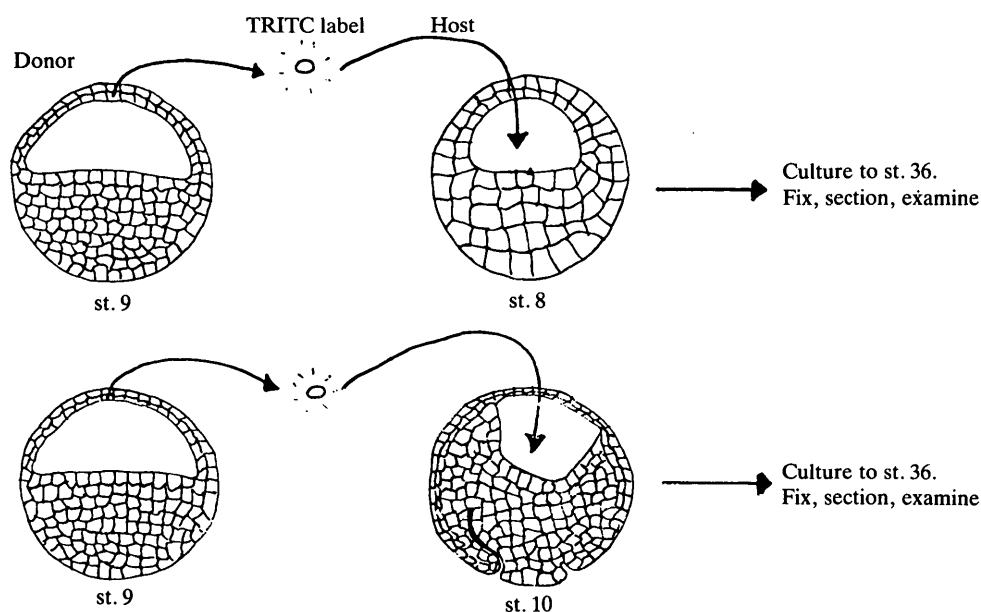


Fig. 9. The experimental design to test the effect of host age on the behaviour of subcortical animal pole cells and their progeny.

blastocoel between. Work is continuing to establish when animal pole cells become irreversibly committed to ectoderm only.

#### *Does cell size play a role in the commitment of cells to particular germ layers?*

One possible and rather trivial explanation of the various results described here is that cell size plays a major role in commitment, as defined by this technique. Thus a very large early blastula cell almost fills the blastocoel and therefore might touch and be influenced by more than one signalling centre of the blastula, resulting in progeny in all germ layers. A cell from an early gastrula is much smaller and as, in

general, it will fall to the floor of the blastocoel, it might only be influenced by the cells here, the prospective endoderm. Two observations argue strongly that this explanation is not a correct one. Firstly, early blastula animal pole cells and midblastula vegetal pole cells are the same size, and yet the animal pole cells are pluripotent while 40 % of vegetal pole cells are committed to form endoderm only. Secondly, the results of culturing experiments argue against cell size being important. When midblastula vegetal pole cells are cultured *in vitro* they divide at a normal rate and by the time sibling embryos have reached early gastrula stage, the progeny *in vitro* are the same size as the vegetal pole cells freshly removed from the early gastrula. Although they are the same size, the cultured cells have a different state of commitment from the gastrula vegetal pole cells (See Table 3).

## CONCLUSIONS

We are still far from being able to describe the mechanism by which cells become committed to particular germ layers. However we can now isolate in pure form, groups of committed or uncommitted vegetal pole cells. This makes possible the comparison at a molecular level of the two states. In particular, the method offers the opportunity to search for differences, perhaps in cell surface molecules, or newly transcribed message. If differences are found then the technology is now available to trace the genes responsible for them. Finally, the techniques described here may be used to manipulate individual blastomeres using specific monoclonal antibodies or gene products, and to alter their state of commitment. Thus, *Xenopus laevis* embryos may become the system of choice for analysing the mechanism of cell commitment in vertebrate development.

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DISCUSSION

*Speaker: Janet Heasman*

*Question from J. Gurdon (Cambridge):*

It seems to me the commitment you talk about could either be that the cells are unable to form particular cell types or that they gradually acquire an ability, perhaps because of their surface, always to stick to a certain point, thereby getting incorporated into certain tissues.

*Answer:*

Yes, determination could be changes in the cell surface which decide where cells go.

*Question from H. Woodland (Warwick):*

First of all, can I comment on that. In our experiments, we find that once cells are committed to form epidermis they normally go to the epidermis after implantation in the blastocoel. However, if you prevent this by putting them deep into the yolk mass, by donor stage 10½ they still form epidermis in the middle of the endoderm.

What I wanted to ask was, as far as endoderm cells are concerned, are you certain that the cells you see are really endoderm? When do ectodermal and mesodermal cells move into that region of the embryo?

*Answer:*

They are all endodermal at the stage of assay.

*Question from I. Dawid (NIH, Bethesda):*

In this experiment where the cells drop to the blastocoel floor – couldn't you turn the egg around and make them drop on the ceiling?

*Answer:*

It's a nice idea. We could do that, but haven't.

*Question from J. McLachlan (St Andrews):*

Do you think your results would be the same if you used a different test for commitment.

*Answer:*

Perhaps not. Until we have genes like segmentation genes, we have to use the techniques which are available and the definitions have to be operational.

*Question from M. Johnson (Cambridge):*

I am not quite clear what you do mean by commitment. When you say 40 %

committed, do you mean one cell is 40 % committed, or 40 % of the cells are fully committed?

*Answer:*

We're trying to work out that precise question by culturing individual cells and analysing what happens to each of the progeny. What we mean by 40 % commitment is that 40 % of the donors give cells only in one germ layer.

*Johnson:*

Isn't your disaggregation experiment with the cultures rather surprising as presumably, at the time of isolation, any one cell is either committed or not? After 2 days culture, you are getting out the same proportion of committed cells.

*Answer:*

There may be a dynamic situation in which a cell can flip between the two possible states. It's the sort of model that is thought to explain differentiation of the melanocytes or blood cells.

*Question from H. Grunz (Essen):*

Can you absolutely exclude a leakage of labelled material from the vegetal pole cells? You may have an endocytosis of this labelled debris by future mesodermal or ectodermal cells.

*Answer:*

We have sectioned embryos just after we have put the cells into the blastocoel to see if that happens, and it doesn't. We have also used cells from *Xenopus borealis* implanted into *Xenopus laevis* and the results are similar.

*Question from Mae Wan Ho (Open University):*

Does your analysis tell us anything about specification?

*Answer:*

Jonathan, you have to define specification.

*J. Slack (ICRF, London):*

Specification is what you measure when you isolate cells in a neutral medium. This is clearly an assay of determination not specification because you are moving the cells to new positions within the embryo and so there will be a variety of significant environmental influences.

*Janet Heasman:*

I have not used the word 'determination' simply because determination tends to suggest an irreversible state. When we can say our committed state is not reversible, then I'll go ahead and call it determined.